Arabinonucleotide Synthesis by the Epoxide Route

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In the search for methods for nucleotide bond formation Todd et al.[1] attempted the phosphorylation method of Bailly[2] based on the epoxide ring opening by sodium phosphates in aqueous solution. The results from the application of this epoxide route to dicarbohydrate esters of phosphoric acid were not encouraging. It proved to be severely limited by the accessibility of appropriate epoxides and the low regioselectivity observed with other than 5,6-anhydrosugar derivatives. This synthetic principle has never been used in oligonucleotide synthesis, since when nucleoside epoxides are used, the latter would produce arabino- or xylo-nucleotides instead of the natural ribo-nucleotides.

The advent of antisense technology[3] has stimulated the design and synthesis of oligoarabinonucleotides as oligoribonucleotide mimics.[4] Meanwhile, 2′,3′-epoxynucleosides of lyxo configuration have become readily available,[5−7] and regioselective opening of epoxides with phosphate anions in aqueous solvent have been reported.[8] These provide the opportunity for testing the epoxide route to arabinonucleotide preparation. Here we report an efficient synthesis of arabinouridylic acid and arabinouridylyl-(3′,5′)-ribouridine using this approach.

When 1-(2′,3′-epoxy-β-D-lyxofuranosyl)uracil[5] (**1**) is reacted with disodium hydrogen phosphate **2**, the HPLC analysis indicates the formation of two products with close retention times (Table 1) in a ratio of approximately 5:1. The major product was isolated by ion-exchange chromatography and subjected to analysis. Enzymic dephosphorylation by alkaline phosphatase afforded arabinouridine (aU). Analysis by ^{1}H and ^{13}C NMR spectroscopy suggests that this product results from lyxo epoxide ring opening by the phosphate dianion to give arabinouridine 3′-phosphate (aUp) **4a** and xylouridine 2′-phosphate (xUp) **4b** as the minor product (Scheme 1). Treatment of the lyxouridine epoxide **1** with disodium uridine 5′-phosphate **3** under the same conditions afforded an improved ratio of the arabino to xylo configuration in the dinucleotide product (Table 1). Assay of the major product **5a** with snake venom phosphodiesterase yielded arabinouridine (aU) and ribouridine 5′-phosphate (prU) **3** while spleen phosphodiesterase yielded arabinouridine 3′-phosphate (aUp) **4a** and ribouridine (rU). Unlike diribonucleoside phosphates, this dinucleoside phosphate does not undergo 2′-hydroxyl-assisted hydrolysis under the reaction conditions used (pH > 7) in accordance with an unfavorable (trans) orientation of the 2′-hydroxyl group. This data coupled with the ^{1}H and ^{13}C NMR spectral data strongly support a highly stereoselective synthesis of an arabinonucleotide bond in arabinouridylyl-(3′,5′)-ribouridine **5a**.

The reaction is much slower (Table 1) when sodium dihydrogen phosphate is used as the nucleophile in accordance with the higher nucleophile reactivity of the phosphate anion ($n = 2$) compared to that of the phosphate dianion ($n = 3.5$).[9,10] A dramatic change of the phosphate anions reactivity, however, is observed when pyridine is used as the solvent (Table 1). The conversion of the nucleoside epoxide is complete after a 6 h reaction time with pyridinium phosphate in pyridine compared to 17% conversion after 12 h with sodium phosphate in water. On the other hand, concurrent hydrolysis in aqueous solution and polymerization in the organic solvent were observed when the phosphate dianion was used as the nucleophile (Table 1). Therefore, pyridine as solvent and pyridinium phosphate as nucleophile afforded an efficient, high-yielding, regio- and stereoselective phosphorylation of lyxouridine epoxide **1** as evidenced by the synthesis of arabinouridylyl-(3′,5′)-ribouridine (aUprU) **5a** (Table 1).

The higher electrophilicity of C3′ compared to C2′ in the lyxo nucleoside epoxides has been observed before in reactions with various nucleophiles[11,12] yet the reason for this difference remains obscure. Recently, the conformation of these epoxides has been established both in the solid state and in solution.[13,14] These lyxoanhydronucleosides were shown to exist predominantly in a O4′-endo puckered conformation or the boat **6** if the six-membered ring C1′−C2′−O2′3′−C3′−C4′−O4′ is considered. Under the neutral or basic conditions used here, the epoxide ring opening follows a S_N2 mechanism.[14] The stereoelectronically controlled transition state in S_N2 reaction and Hammod postulate, applied to the epoxide ring opening, require that the reaction at C3′ of the conformationally rigid epoxide must give arabino product in S(3′-exo,2′-endo) puckered conformation **7a**/**8a** whereas the reaction at C2′ must give the product in N(3′-endo,2′-exo) puckered conformation **7b**/**8b** (see Scheme 2). We tentatively assume the formation of the transition state to produce **7a** to be a lower energy process than the one leading to **7b**. Actually, the former could be stabilized by H-bonding of the leaving oxyion with the axial hydroxymethyl group. Therefore, the arabino product **4a**/**5a** is formed in preference to the xylo product **4b**/**5b** under kinetically controlled conditions. This interpretation is fully supported by the increased selectivity in the organic solvent (pyridine) (Table 1) that would favor the electrostatic interactions: the reaction of phosphate ion is over within 6 h

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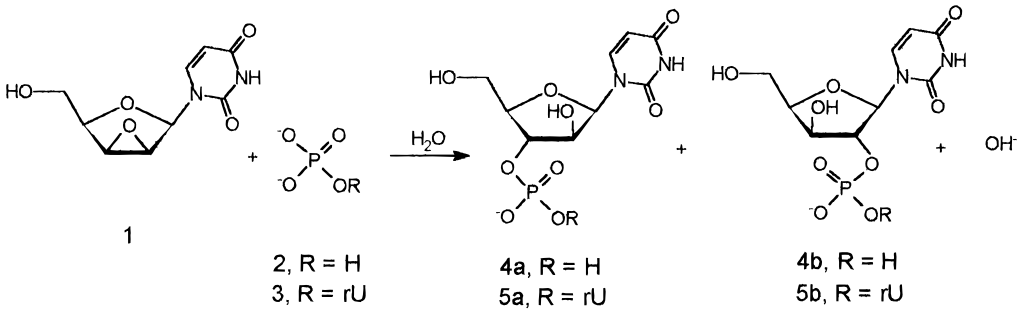
Table 1. Reaction Parameters of the Lyxoepoxide Ring Opening by Phosphate and Uridine 5′-Phosphate Anions at 70 °C

Product(s)	Reaction conditions: Solvent	Nucleophile	Reaction time (h)	Total yield (%)	Isomeric ratio
aUp/xUp (4a/4b)	water	$2Na^+$ ^-O_3PO	12	64	4.2/1
		Na^+ $HO(O^-)PO$	12	17	4.5/1
	pyridine	$2Bu_4N^+$ ^-O_3PO	4	53	8.6/1
		$PyrH^+$ $HO(O^-)PO$	6	98	8.0/1
aUpU/xUpU (5a/5b)	water	$2Na^+$ ^-O_3PO-rU	24	87	9.3/1
	pyridine	$PyrH^+$ $HO(O^-)PO$-rU	6	96	12.0/1

Scheme 1



1

2, R = H
3, R = rU

4a, R = H
5a, R = rU

4b, R = H
5b, R = rU

in pyridine and is in the beginning (17% conversion) in water. Moreover, a much lower selectivity has been observed[11] in the absence of the 5′-hydroxyl group in lyxoadenosine epoxide ring opening with different nucleophiles.

In conclusion, this report demonstrates the preparation of arabinonucleotides using a new synthetic principle: the formation of the nucleotide bond by O−C instead of the usual P−O bond formation in a reaction of a nucleoside epoxide with a phosphate anion. It will benefit the oligoarabinonucleotide synthesis as the preparation of nucleotide mimetics in antisense technology.

Experimental Section

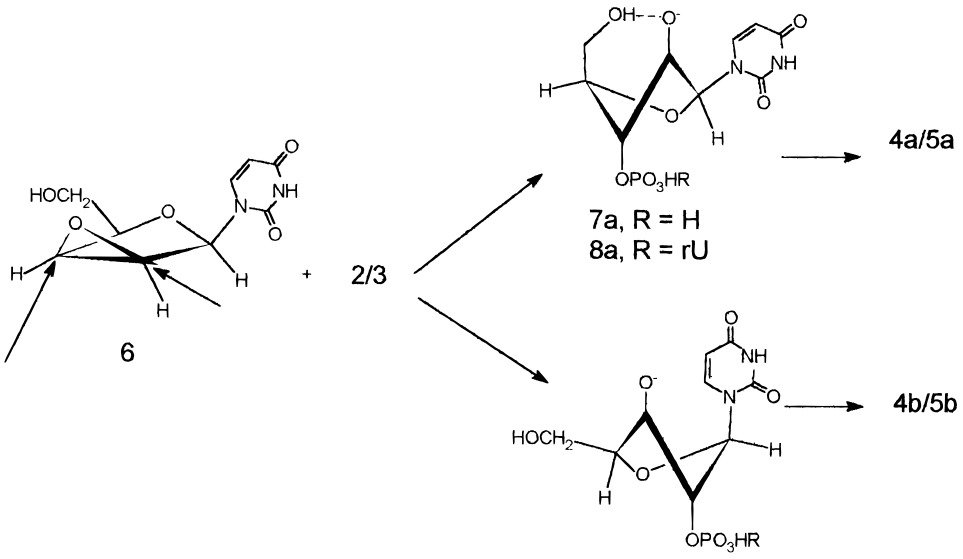
General Procedure for Lyxoepoxide Ring Opening of Lyxoepoxide Uridine by Phosphate Anions. Equimolar amounts (0.17 mmol) of 1-(2′,3′-epoxy-β-D-lyxofuranosyl)uracil, prepared as described by Codington et al.,[5] and the corresponding phosphate or uridine 5′-phosphate (BIOSYNTH, Germany) were dissolved in 2 mL of water or pyridine dried over KOH. The reaction mixture was stirred at 70 °C, and the progress of the reaction was followed by HPLC using an amino column (Spherisorb-NH_2, 5 μm, 250 × 4.6 mm), isocratic elution with 50 mM KH_2PO_4/acetonitrile(70/30 v/v, pH 4.6), flow rate 1 mL/min, and detection wavelength 280 nm. After the reaction was over, the solvent was evaporated, and the residue was chromatographed on a 250 × 10 mm (i.d.) DEAE-cellulose column (OH^--form), stepwise gradient elution with 0−0.1 M NH_4HCO_3 using Pharmacia Standard Chromatography System II and detection wavelength 254 nm. The corresponding fractions were pooledand evaporated to dryness, and the residue was subjected to enzymatic and NMR ^1H and ^{13}C NMR analysis.

NMR Spectroscopic Analysis. 1D and 2D and ^1H and ^{13}C NMR spectra were recorded on JEOL-Lambda 400 and BRUKER Avance-DRX 250 spectrometers. The confirmative assignments were obtained by using 1D ROED and 2D $^1H-^1H$ COSY, HMOC, NMBC, and NOESY experiments.

Arabinouridine 3′-phosphate (aUp) 4a: ^1H NMR (D_2O, 25 °C, 400 MHz, HDO ($\delta = 4.84$)): $\delta = 3.81$ (dd, $J = 12.5$, 5.3 Hz, 1H, H-5′), 3.90 (dd, $J = 12.5$, 3.5 Hz, 1H, H-5′), 3.97 (m, 1H, H-4′), 4.11 (dd, $J = 5.4$, 4.7 Hz, 1H, H-2′), 4.40 (dd, $J = 4.8$, 4.5 Hz, 1H, H-3′), 5.87 (d, $J = 7.5$ Hz, 1H, H-5), 6.17 (d, $J = 5.0$ Hz, 1H, H1′), 7,84 (d, $J = 7.8$ Hz, 1H, H-6); $^{13}C\{H\}$ NMR (D_2O, 25 °C, 100 MHz, TSPA ($\delta = 0$), as pyridinium salt): $\delta = 61.99$ (s, C-5′), 78.44 (s, C-3′), 79.01 (s, C-2′), 82.25 (s, C-4′), 87.98 (s, C-1′), 104.91 (s, C-5), 144.82 (s, C6), 150.32 (s, C-2), 164.42 (s, C-4). Anal. Calcd for $C_9H_{13}N_2O_9P$ (324.18): C, 33.35; H, 4.04; N, 8.64. Found: C, 33.59; H, 4.27; N, 8.71.

Arabinouridylyl-(3′,5′)-ribouridine (aUprU) 5a: ^1H NMR

Scheme 2



(D_2O, 25 °C, 400 MHz, HDO ($\delta = 4.84$)): $\delta = 3.85$ (dd, $J = 12.6$, 6.5 Hz, 1H, H-5′ of aU), 3.92 (dd, $J = 12.0$, 3.5, 1H, H5′ of aU), 4.12 (m, 1H, H-5′ of rU), 4.19 (m, 1H, H-5′ of rU), 4.21 (m, 1H, H-4′ of aU), 4.27 (m, 1H, H-4′ of rU), 4.34 (dd, $J = 9.7$, 4.8 Hz, 1H, H-3′ of rU), 4.37 (dd, $J = 9.7$, 4.5 Hz 1H, H2′ of rU), 4.51 (m, 2H, H-2′, H-3′ of aU), 5.86 (d, $J = 8.5$ Hz, 1H, H-5 of aU), 5.92 (d, $J = 9.3$ Hz, 1H, H-5 of rU), 5.96 (d, $J = 4.3$ Hz, 1H, H-1′ of rU), 6.16 (d, $J = 2.0$ Hz, 1H, H-1′ of aU), 7.8 5 (d, $J = 8.5$ Hz, 1H, H-6 of aU), 7.90 (d, $J = 9.3$ Hz, 1H, H-6 of rU); $^{13}C\{H\}$ NMR (D_2O, 25 °C, 100 MHz, TSPA ($\delta = 0$)): $\delta = 63.62$ (s, C-5′ of aU), 67.58 (d, $^2J(P,C) = 5.1$ Hz, C-5′ of rU), 72.34 (s, C-3′ of rU), 76.51 (s, C-2′ of rU), 77.12 (d, $^3J(P,C) = 4.5$ Hz, C-2′ of aU), 82.19 (d, $^2J(P,C) = 5.0$ Hz, C-3′ of aU), 85.67 (d, $^3J(P,C) = 8.4$ Hz, C-4′ of rU), 86.30 (d, $^3J(P,C) = 5.0$ Hz, C-4′ of aU), 88.72 (s, C-1′ of aU), 91.87 (s, C-1′ of rU), 103.82 (s, C-5 of aU), 105.30 (s, C-5 of rU), 144.59 (s, C-6 of rU), 145.97 (s, C-6 of aU), 154.11 (s, C-2 of aU), 154.56 (s, C-2 of rU), 168.96 (s, C-4 of aU), 169.10 (s, C-4 of rU). Anal. Calcd for $C_{18}H_{23}N_4O_{14}P\cdot 1.5H_2O$ (577.39): C, 37.45; H, 4.54; N, 9.70. Found: C, 37.83; H, 4.61; N, 9.68.

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Additions and Corrections

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Jennifer A. Sowinski and Peter L. Toogood*. Synthesis of an Enantiomerically Pure Serine-Derived Thiazole.

Page 7671. Optical rotation values for compounds **7** and **8** were inadvertantly misreported. The correct values are as follows: **7** $[\alpha]^{25}_D = +34.4$ (*c* 1, $CHCl_3$); **8** $[\alpha]^{25}_D = +65.0$ (*c* 1, $CHCl_3$).

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